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GRANT NUMBER DAMD17-97-1-7187

TITLE: The Role of Phosphotyrosine Phosphatases in Breast Cancer

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REPORT DATE: June 1999

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 1999	Final (1 Jun 97 - 31 May 99)

4. TITLE AND SUBTITLE

The Role of Phosphotyrosine Phosphatases in Breast Cancer

5. FUNDING NUMBERS

DAMD17-97-1-7187

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Indiana University
Bloomington, Indiana 47402-1847

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

This report describes the development and synthesis and studies of a biotinylated affinity reagent of Yop51. This molecule is being used to isolate Yop51 that is present in *E. coli* cell lysate.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Julie Rostamadji 6/19/99

PI - Signature Date

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INTRODUCTION

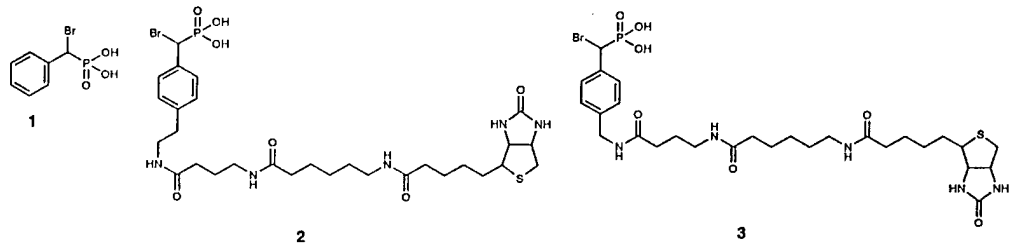
The increased expression of certain phosphotyrosine phosphatases (PTPases) has been associated with oncogenic cell transformation in a number of different tissues, including breast tissue. The exact role of PTPases in transformed cells is still not yet determined, but it is likely that they play a key role in tumorigenesis. Therefore, PTPases have become a topic of interest in signal transduction research. In our laboratory, we are interested in learning more about PTPases and their possible roles in tumorigenesis. *Yersinia* PTPase (Yop51) is the enzyme we work with. It shares a significant homology to most members of PTPase enzyme family. In the previous report, the synthesis and studies of the prototypic, nonpeptidic affinity reagent for PTPases were described. Along with that on-going effort, we are in the process of developing biotinylated affinity reagents to "fish out" PTPases from cell lysate. Such molecules may potentially serve as tools to identify PTPases that are expressed in tumor cells.

BODY

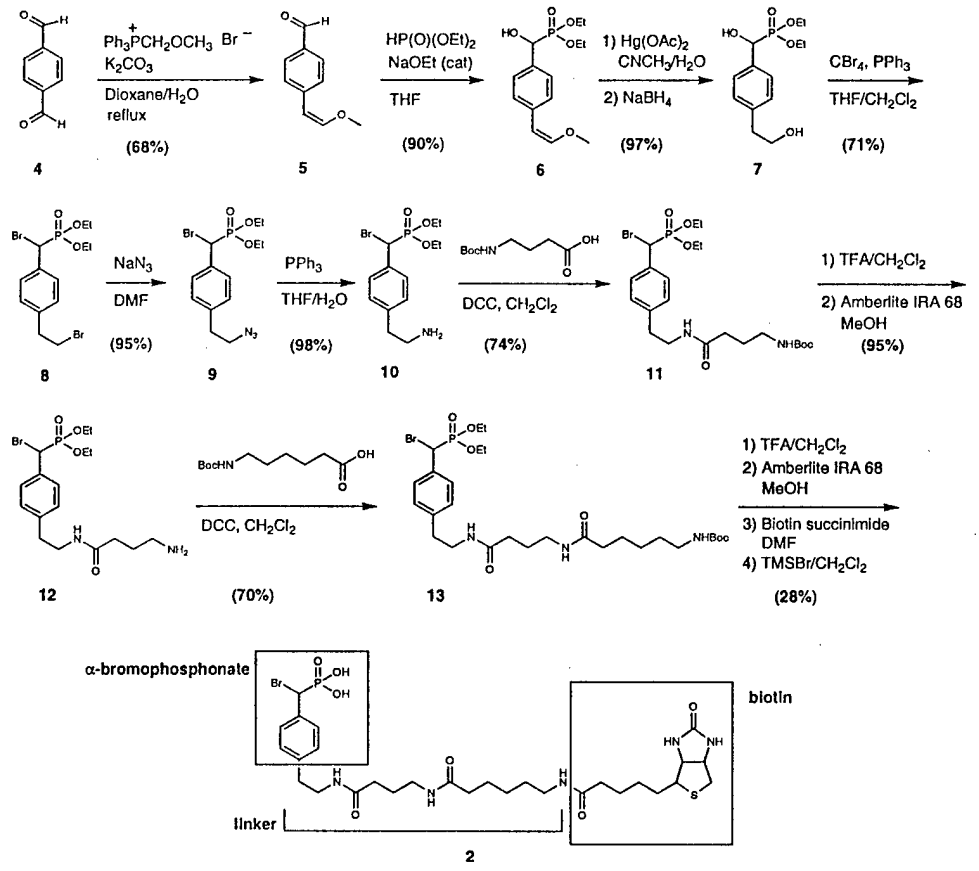
Biotinylated Analog of a-Bromobenzylphosphonate (2)

We incorporated the prototype affinity reagent (compound **1**) into a biotinylated analog (compound **2**) as shown in the synthetic scheme 1. Compound **2** comprises of three components, the α-bromophosphonate, a linker and a biotin molecule. The ability of compound **2** to inactivate Yop51 is comparable to compound **1**. Their IC50 values are 1.8 mM and 1.9 mM respectively. Compound **2** was designed such that the α-bromobenzylphosphonate moiety covalently modifies Yop51. The linker would expose the biotin portion into solution. The labeled enzyme could then be probed using streptavidin-horseradish peroxidase conjugate and detected using chemiluminescent reagents.

Figure 1. Affinity Reagents for PTPases



Scheme 1. Synthetic scheme of compound **2**



Labeling of Yop51

The enzyme was inactivated with a range of concentrations of compound **2**. The mixtures were then loaded on to a SDS Page gel. The protein bands were subsequently transblotted on to a polyvinylidene fluoride (PVDF) microporous membrane. The membrane was treated with blocking agent (5% low fat dry milk in phosphate saline buffer) and probed with streptavidin-horseradish peroxidase conjugate. ECL Western blotting reagents were used to identify the bound streptavidine conjugate. The chemiluminescent reaction was detected by exposure to blue-light sensitive autoradiography film.

Compound **3** (Scheme 2) was prepared as a control molecule to ensure that Yop51-compound **2** adduct is formed due to the inactivation of the enzyme by the α-bromophosphonate moiety. Compound **3** is similar to compound **2** except for the absence of bromide at the α-position. It has a shorter linker than compound **2**. Our preliminary studies (not shown here) indicated any linker comprised of no less than 12 atoms is able to place the biotin moiety at the surface of the protein to interact with streptavidin conjugate.

Scheme 2. Synthetic scheme of compound **3**

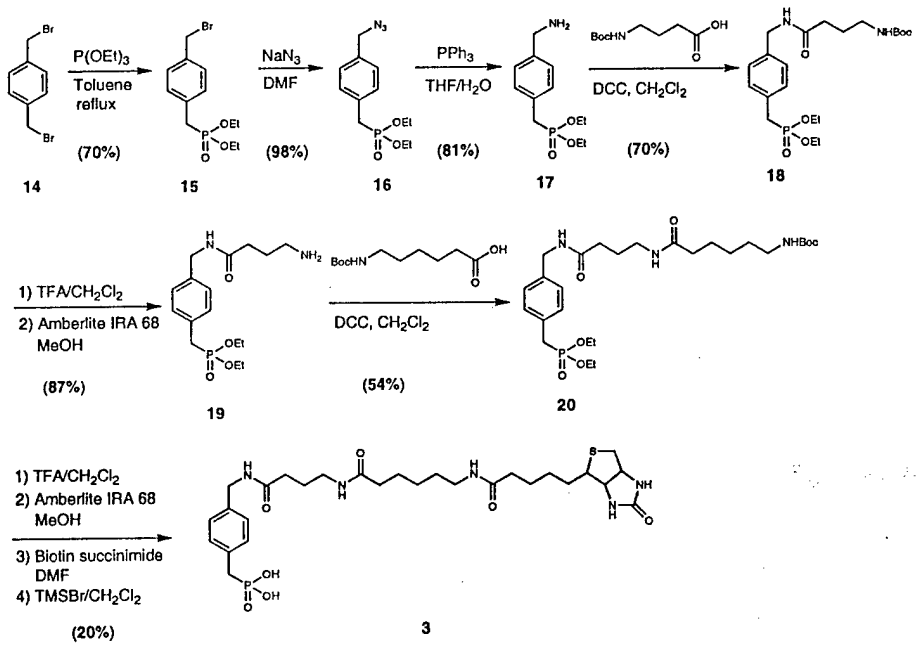
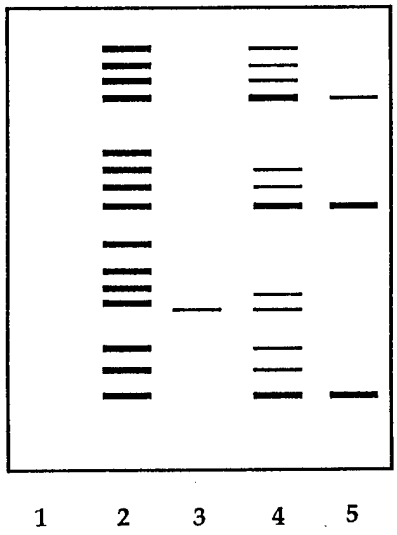


Figure 2. Representation of labeled proteins detected on autoradiography film
Lane 1: molecular weight marker; lane 2: 200 μg protein from E. coli cell lysate treated with 0.05 mM compound **2**; lane 3: 1.75 μg Yop51 treated with 0.05 mM compound **2**; lane 4: a mixture of 1.75 μg Yop51 and 100 μg protein from E. coli cell lysate treated with 0.05 mM compound **2**; lane 5: 100 μg protein from E. coli cell lysate treated with 0.05 mM compound **3**.



Within detection limits, labeled Yop51 was observed when it was treated with 0.05 mM of compound **2** at minimum. Compound **3**, on the other hand, gave no detectable protein adduct at this concentration (data not shown).

Indicated in Figure 2, treating *E. coli* cell lysate (200 μg total protein concentration) with 0.05 mM of compound **2** revealed that compound **2** labeled a large number of proteins (lane 2). We are interested to determine the nature of modification of those proteins by compound **2**. Labeled Yop51 (1.75 μg total protein concentration) gave a single band (lane 3). Treating a mixture of 100 μg *E. coli* cell lysate and 1.75 μg Yop51 with 0.05 mM of compound **2** gave a number of labeled proteins (lane 4). Unfortunately it is not clear whether Yop51 was labeled or not since its band coincides with an *E. coli* protein of similar molecular weight.

CONCLUSION

Our effort to develop a biotinylated affinity reagent that can be used to isolate PTPases in tumor cells has resulted in the synthesis and studies of compound **2**. It inactivates Yop51 in low millimolar range and the labeled protein can be detected by probing the biotin moiety with streptavidin-horseradish peroxidase conjugate. Further studies are being carried out to identify labeled Yop51 in the presence of other proteins present in cell extract.

APPENDIX

Key Research Accomplishments

* Synthesis and studies of non-peptidic inactivators of Yop51

* Synthesis and studies of biotinylated affinity reagent of Yop51

* Isolation and detection of Yop51 labeled with the biotinylated affinity reagent